ABRF 2017 ANNUAL MEETING

SAN DIEGO CA, USA

MARCH 25 – 28, 2017 | TOWN AND COUNTRY RESORT & CONVENTION CENTER

A FORUM FOR ADVANCING TODAY'S CORE TECHNOLOGIES TO ENABLE TOMORROW'S INNOVATIONS

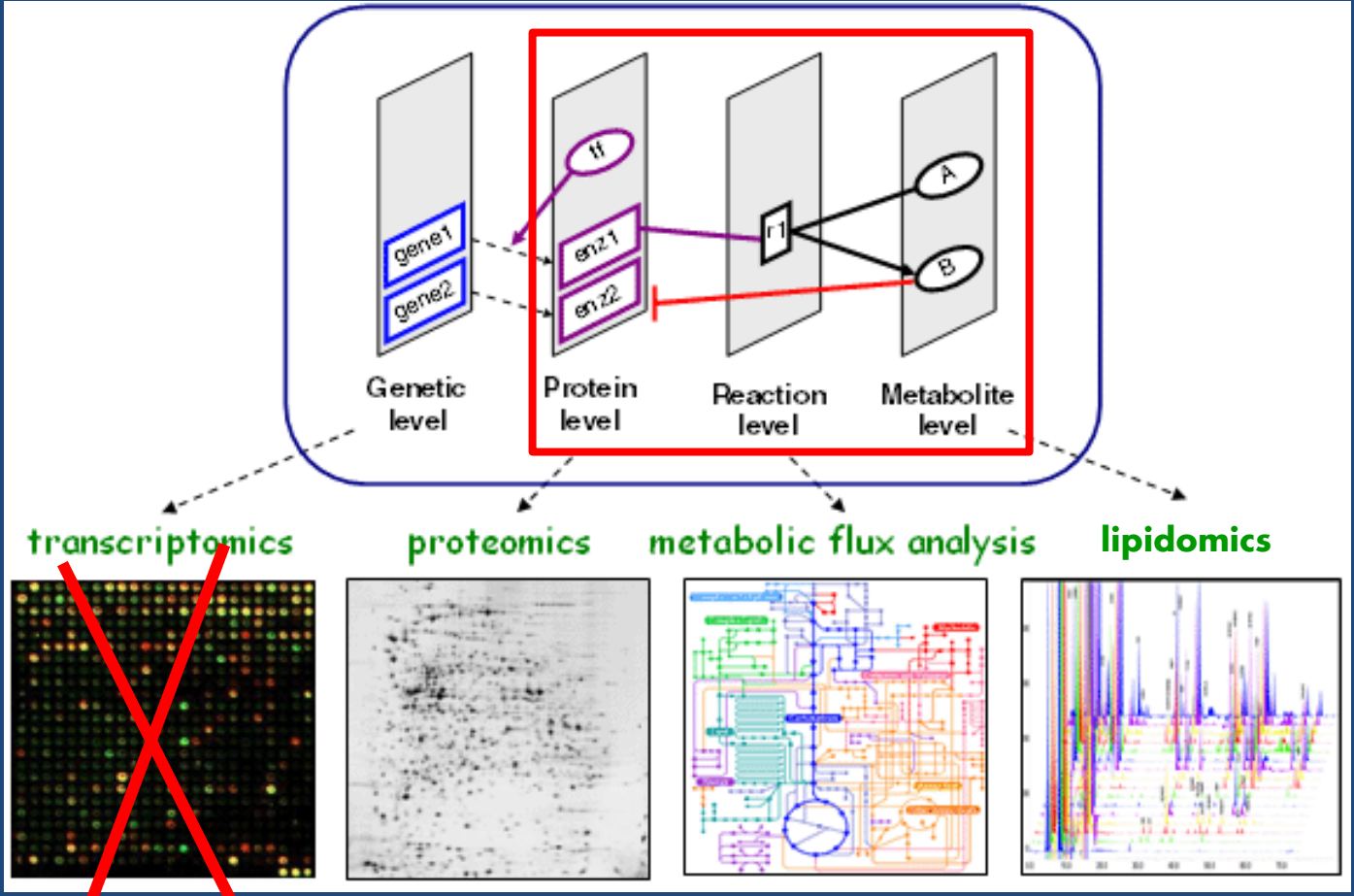
Lipidomics in Systems Biology – Untargeted Pos/Neg Switching LC-MS/MS Platform

John M. Asara, Ph.D.
Beth Israel Deaconess Medical Center
Harvard Medical School

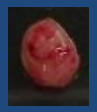
Lipidomics Workshop
03/25/2017

Cancer Center | Beth Israel Deaconess Medical Center | HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

Integrating Different –*Omics* Approaches can Reveal insight into Biological Systems of Disease



Serial-Omics (Lipidomics) workflow



MTBE extraction

Top Layer: Non-polar Lipids

Middle Layer: Polar Metabolites

Pellet: Protein, DNA

Non-polar Lipids

Speed-Vac Dry
Resuspend in 50/50 IPA/MeOH

DDA, HCD, +/- switch, RP-C18

LipidSearch software
Elements software
MetaboAnalyst

Polar Metabolites

Speed-Vac Dry
Resuspend in H_2O

SRM, **+/-** switch, 300 unlabeled targets
185 $^{13}C/^{15}N$ targets, Amide-HILIC

MultiQuant
MetaboAnalyst

DDA, HCD, +/- switch

Elements

Peptides/Phosphopeptides

Pellet dissolved in
lysis buffer

Digest with Trypsin
Fractionation
IMAC (Fe^{3+})
TMT labeling (126, 128)

DDA, HCD, RP-C18

Mascot
Scaffold
MaxQuant

Fatty Acid Synthesis

Lipid Arrays

Glycolysis → TCA cycle → Acetyl-coA

ACC

Malonyl-coA

FAS

Palmitate (C16:0) —**SCD1 desaturation**→ Palmitoleate (C16:1) →

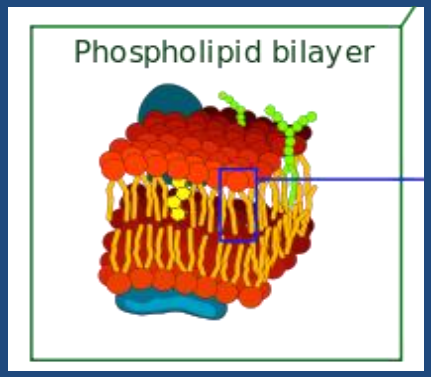
- increase insulin sensitivity (high conc. in liver)
- inhibit the destruction of insulin-secreting pancreatic beta cells

Palmitate (C16:0) —**elongation**→ Stearate (C18:0) —**SCD1 desaturation**→ Oleate (C18:1) → TG

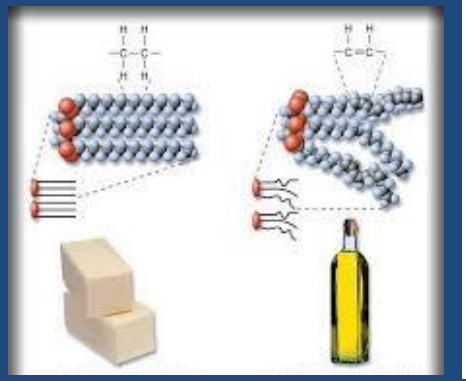
- Mostly present as triglycerides (olive oil, animal fat, etc.)

Stearate (C18:0) —**elongation**→ Arachidate (C20:0) —**desaturation**→ Eicosenoate (20:1) →

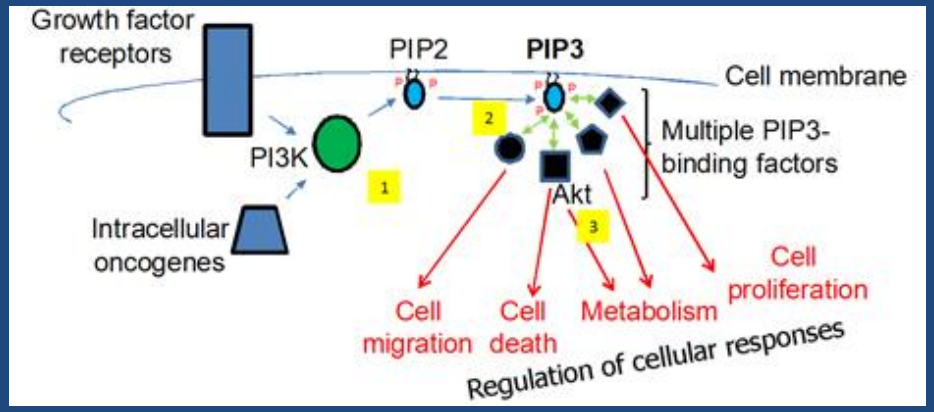
Membrane structure



Fats



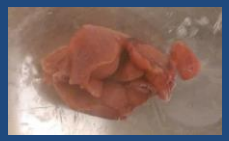
Signaling Lipids



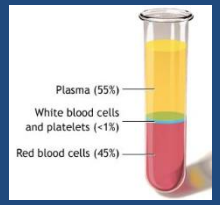
Platform for *Untargeted* Lipidomics

Cancer cells

Tumor tissue

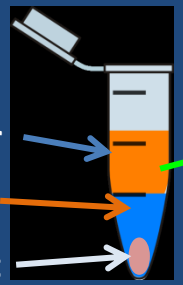


Blood plasma



flies

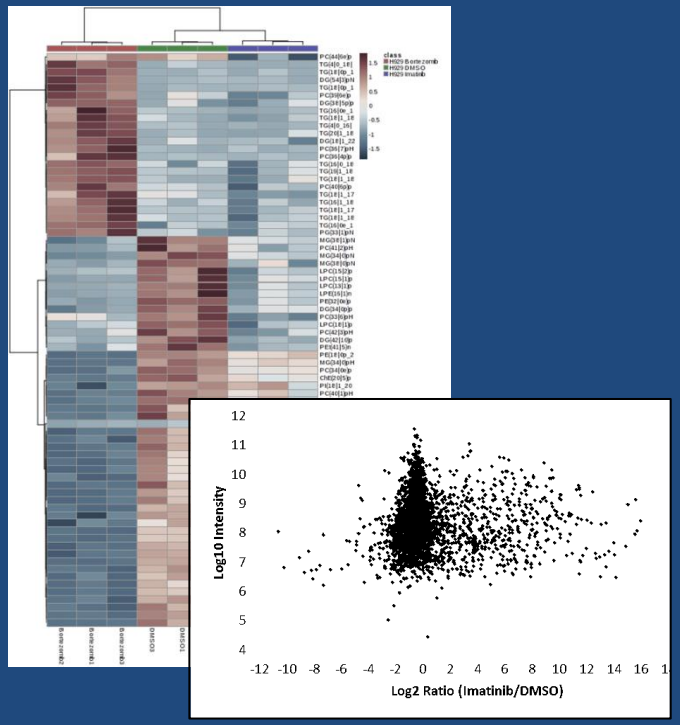
methyl-tert-butyl ether (MTBE)

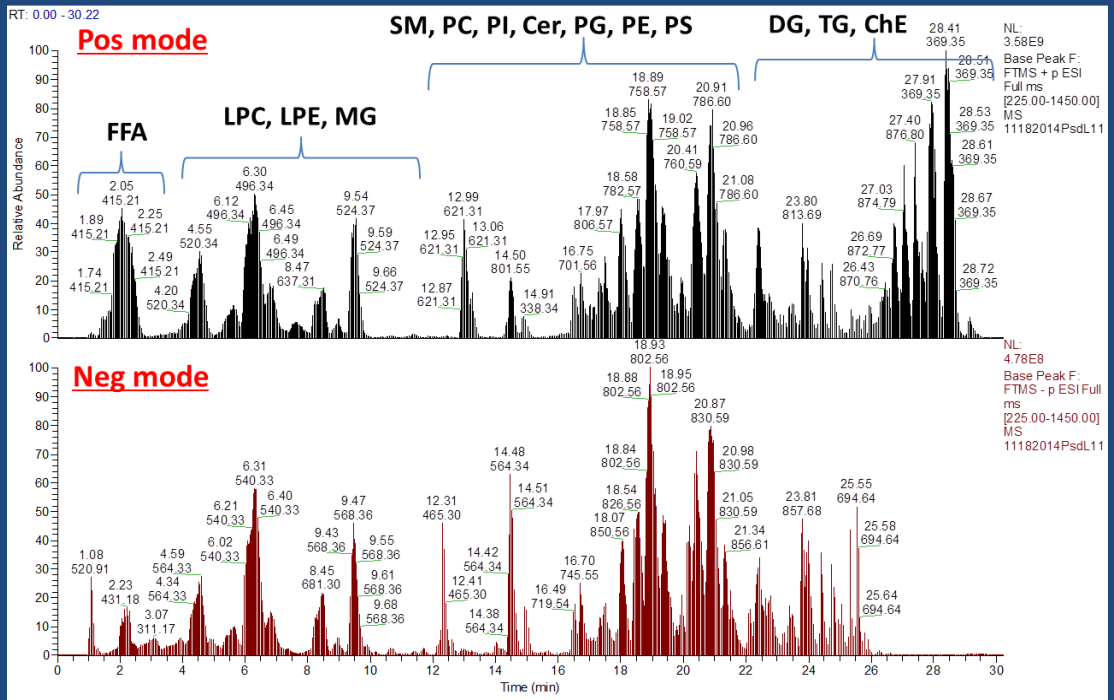


Thermo QExactive Plus/HF Agilent 1100/1200

2.1 mm x 10 cm C_{18} (low pH, high organic)
260 µL/min

Pos/Neg polarity switching (~10 points/peak)
HCD-DDA (Top 8) pos *and* neg mode

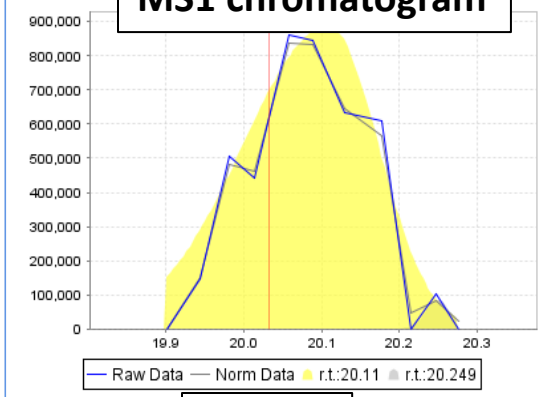




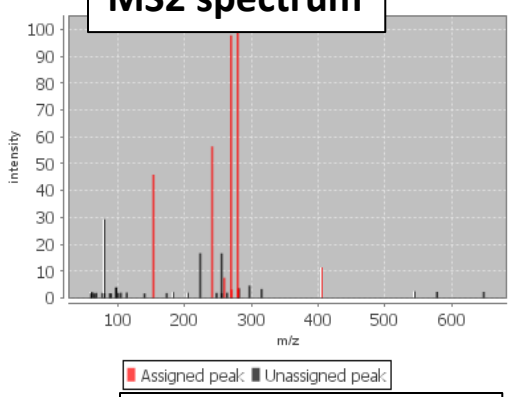
Breitkopf et al, *Metabolomics*, 2017

Thermo LipidSearch MS2 Based Identification Process

MS1 chromatogram



MS2 spectrum



Scoring

Match Lipid

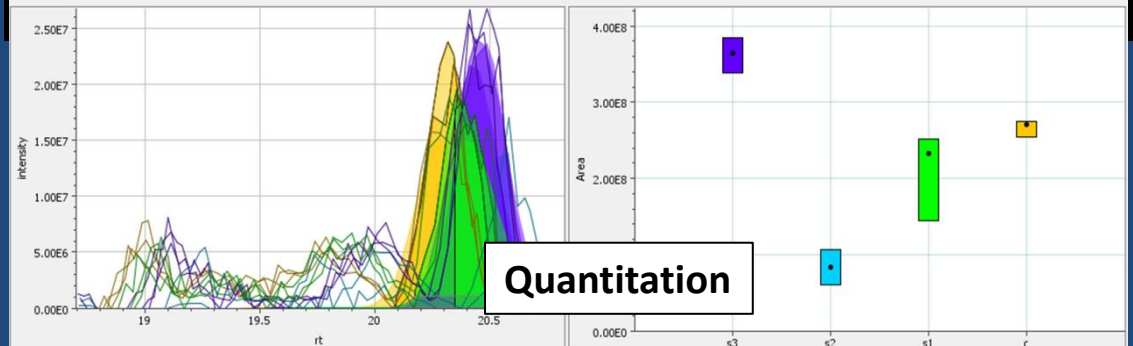
LipidIon	M-Sc.	T-Sc.	Sc.	St.
PI(17:0/18:2)-H	43.3	0.4	72.2	
PI(18:2/17:0)-H	43.3	0.4	72.2	
PI(16:0/19:2)-H	11.2	0.4	28	
PI(19:2/16:0)-H	11.2	0.4	28	
PI(17:1/18:1)-H	10.1	0.4	25.2	
PI(18:1/17:1)-H	10.1	0.4	25.2	
PI(11:0/24:2)-H	7.3	0.4	24.4	
PI(13:0/22:2)-H	7.3	0.4	24.4	
PI(15:0/20:2)-H	7.3	0.4	24.4	
PI(15:1/20:1)-H	7.3	0.4	24.4	
PI(16:1/19:1)-H	7.3	0.4	24.4	

HCD fragmentation

Match Detail

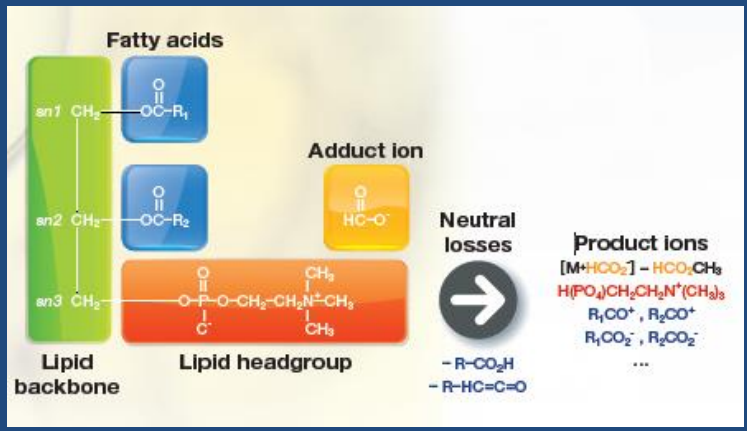
ObsMz	Type	It.(%)	Frag.	Delta(Da)
152.9949	MS2	45.906	GP-H3O	-0.0009
172.7457	MS2	1.882	-	-
182.1563	MS2	2.169	-	-
204.3326	MS2	2.012	-	-
223.0009	MS2	16.663	-	-
241.0119	MS2	56.445	PH(inositol)-H	0
247.5671	MS2	1.936	-	-
255.1215	MS2	1.828	-	-
255.2328	MS2	16.623	-	-
259.0225	MS2	7.551	IP	0.0001
263.2816	MS2	2.008	-	-
269.249	MS2	97.74	FA(17:0)-H	0.0004
270.2529	MS2	3.21	FA(17:0)-H [is	1.0043
279.2333	MS2	100	FA(18:2)-H	0.0003

Quantitation



- Untargeted identification based on *FRAGMENTATION and high mass accuracy MS and MS2 data*

Phospholipid



- Capable of identifying >1500 lipid ions in 30 min. with pos/neg switching from 66 subclasses of lipids
- Database assisted de novo interpretation

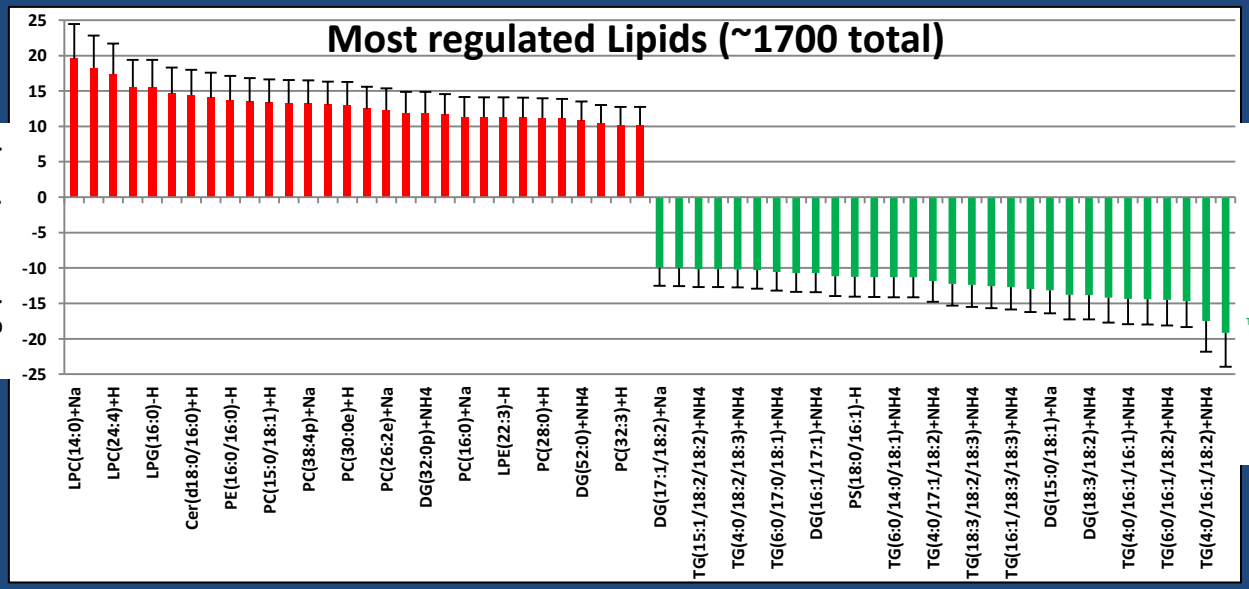
18 lipid classes and 66 subclasses can be identified via LC-MS/MS and LipidSearch

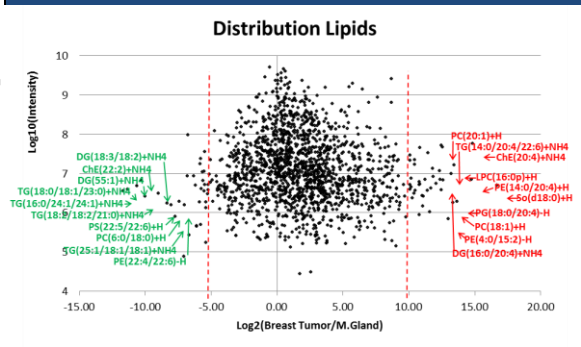
Lipid Family	Lipid Class	Abr.
P-Choline	lysophosphatidylcholine	LPC
	platelet-activating factor	PAF
	phosphatidylcholine	PC
P-Ethanol Amine	lysophosphatidylethanolamine	LPE
	lysodimethylphosphatidylethanolamine	LdMePE
	phosphatidylethanolamine	PE
	dimethylphosphatidylethanolamine	dMePE
P-Serine	lysophosphatidylserine	LPS
	phosphatidylserine	PS
P-Glycerol	lysophosphatidylglycerol	LPG
	phosphatidylglycerol	PG
P-Inositol	lysophosphatidylinositol	LPI
	phosphatidylinositol	PI
	phosphatidylinositol	PIP
	phosphatidylinositol	PIP2
	phosphatidylinositol	PIP3
P-Ethanol	lysophosphatidylethanol	LPEt
	phosphatidylethanol	PEt
P-Acid	lysophosphatidic acid	LPA
	phosphatidic acid	PA
	cyclic phosphatidic acid	cPA
P-Methanol	lysophosphatidylmethanol	LPMe
	phosphatidylmethanol	PMe
Sphingolipids	sphingomyelin	SM
	sphingomyelin(phytosphingosine)	phSM
Neutral glycerolipid	monoglyceride	MG
	diglyceride	DG
	triglyceride	TG
Fatty Acid	fatty acid	FA
	(O-acyl)-1-hydroxy fatty acid	OAHFA
Cardiolipin	Cardiolipin	CL
Sphingoid base	Sphingoshine	So
	Sphingoshine phosphate	SoP
Glycosphingolipids	Ceramides	Cer
	Ceramides phosphate	CerP
	Gangliosides	GM3
	Gangliosides	GM2
	Gangliosides	GM1
	Gangliosides	GD1a
	Gangliosides	GD1b
	Gangliosides	GD2
	Gangliosides	GD3
	Gangliosides	GT1a
	Gangliosides	GT1b
	Gangliosides	GT1c
	Gangliosides	GT2
	Gangliosides	GT3
	Gangliosides	GQ1c
	Gangliosides	GQ1b
Neutral Glycosphingolipids	Simple Glc series	CerG1
	Simple Glc series	CerG2
	Simple Glc series	CerG3
	Simple Glc series	CerG2GNAc1
	Simple Glc series	CerG3GNAc1
	Simple Glc series	CerG3GNAc2
Steroid	Cholesteryl Ester	ChE
	zymosteryl	ZyE
	Stigmasteryl ester	StE
	Sitosteryl ester	SiE
Coenzyme	Coenzyme	Co
Glycoglycerolipid	Monogalactosylmonoacylglycerol	MGMG
	Monogalactosyldiacylglycerol	MGDG
	Digalactosylmonoacylglycerol	DGMG
	Digalactosyldiacylglycerol	DGDG
	Sulfoquinovosylmonoacylglycerol	SQMG
	Sulfoquinovosyldiacylglycerol	SQDG

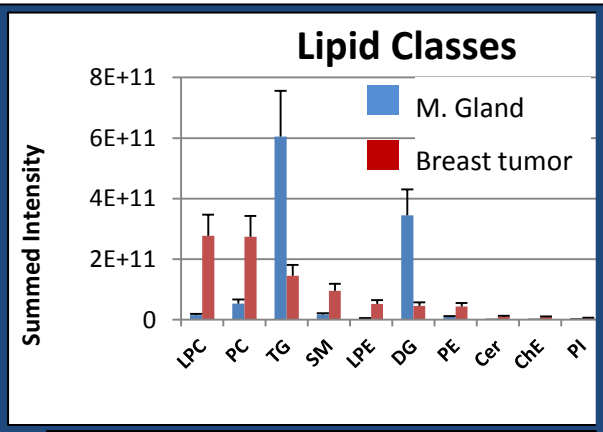
Output breaks down the lipid structure

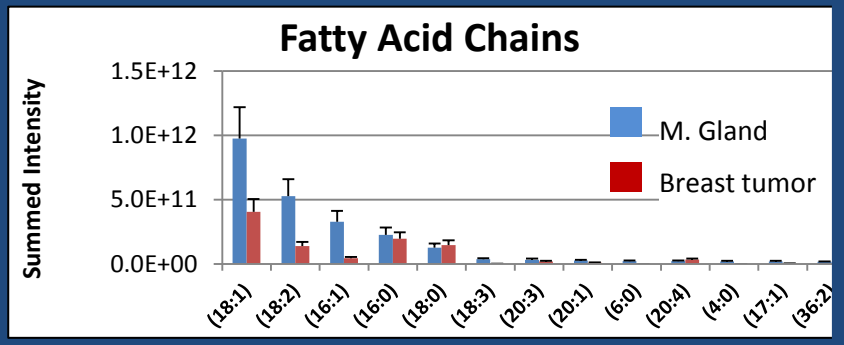
LipidIon	LipidGroup	Class	FattyAcid	FA1	FA2	FA3	FA4	CalcMz	IonFormula
CL(14:0/16:0/16:0/16:1)-H	CL(62:1)-H	CL	(14:0/16:0/16:0/16:1)	(14:0)	(16:0)	(16:0)	(16:1)	1321.918005	C71 H135 O17 P2
CL(14:0/16:0/16:1/16:1)-H	CL(62:2)-H	CL	(14:0/16:0/16:1/16:1)	(14:0)	(16:0)	(16:1)	(16:1)	1319.902355	C71 H133 O17 P2
CL(14:0/16:1/16:1/16:1)-H	CL(62:3)-H	CL	(14:0/16:1/16:1/16:1)	(14:0)	(16:1)	(16:1)	(16:1)	1317.886705	C71 H131 O17 P2
CL(14:0/16:0/16:0/18:1)-H	CL(64:1)-H	CL	(14:0/16:0/16:0/18:1)	(14:0)	(16:0)	(16:0)	(18:1)	1349.949305	C73 H139 O17 P2
CL(14:0/16:1/16:0/18:1)-H	CL(64:2)-H	CL	(14:0/16:1/16:0/18:1)	(14:0)	(16:1)	(16:0)	(18:1)	1347.933655	C73 H137 O17 P2
CL(14:0/16:1/16:1/18:1)-H	CL(64:3)-H	CL	(14:0/16:1/16:1/18:1)	(14:0)	(16:1)	(16:1)	(18:1)	1345.918005	C73 H135 O17 P2
CL(16:1/16:1/16:1/16:1)-H	CL(64:4)-H	CL	(16:1/16:1/16:1/16:1)	(16:1)	(16:1)	(16:1)	(16:1)	1343.902355	C73 H133 O17 P2
CL(16:1/16:1/16:1/16:1)-2H	CL(64:4)-2H	CL	(16:1/16:1/16:1/16:1)	(16:1)	(16:1)	(16:1)	(16:1)	671.447539	C73 H132 O17 P2

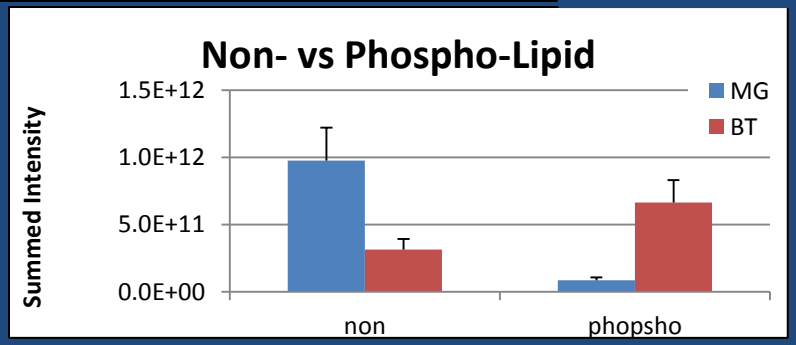
Lipidomics LipidSearch output from 10 mg Breast Tumor vs. Mammary Gland





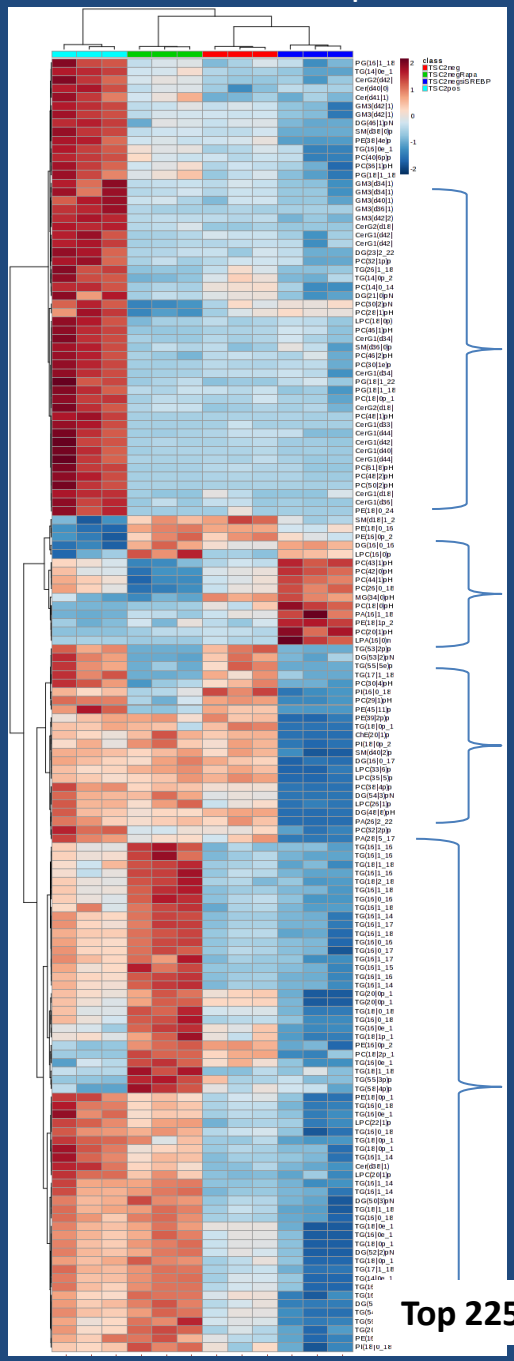






- **Phospholipids are most enriched in breast tumor (PC, PE, LPC, LPE, etc.)**
- **Triglycerides/diglycerides are enriched in breast mammary gland**

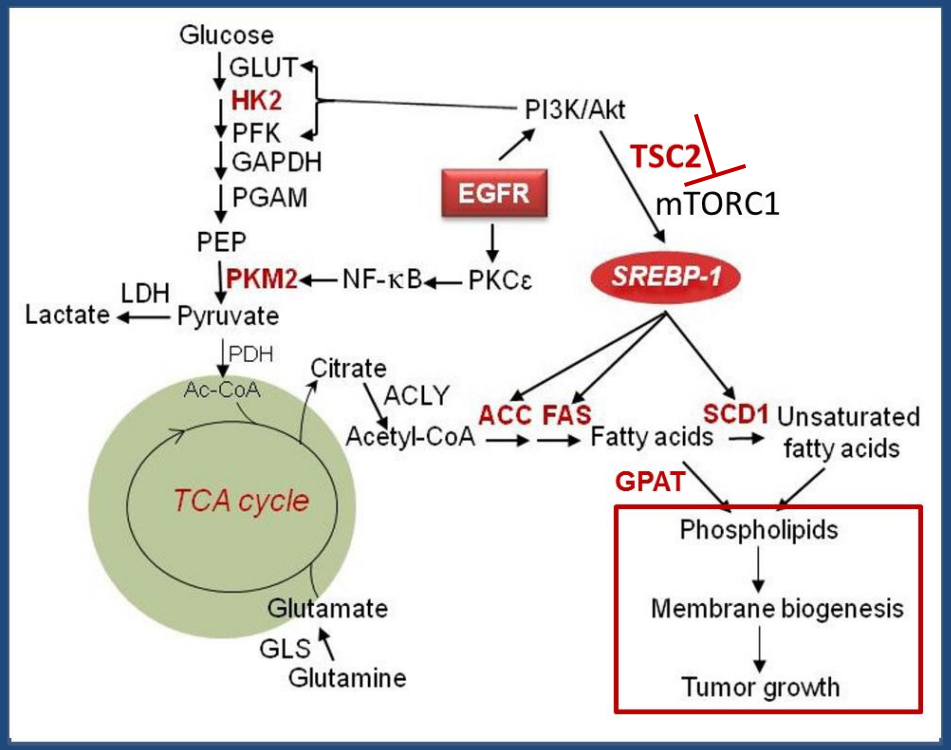
Clustering of TSC2 Lipidomics LipidSearch Data using *MetaboAnalyst*



Biological replicates
$R^2 > 0.95$

Top 225/>2,000 lipids

De Novo Lipid Synthesis Pathway



Ru et al, Cancers 2013, 5(4), 1469-1484

Breitkopf et al, *Metabolomics*, 2017

Untargeted Metabolomics/Lipidomics using *Elements* Spectral Library Matching Software (Proteome Software)

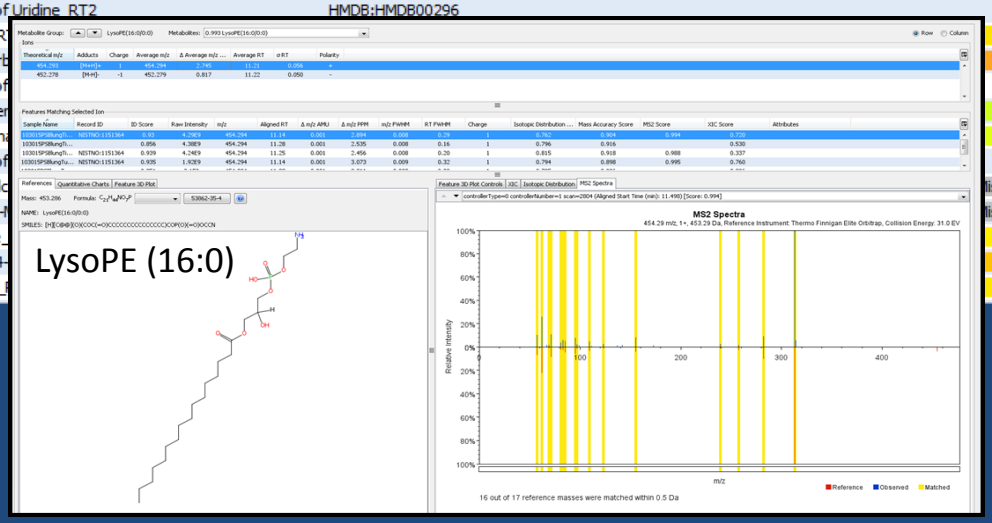
Log10 Intensity

Color Legend (Displayed Value)

9.93
9.13
8.32
7.52
6.71

>550 identified metabolites (NIST & HMDB)

ID Score	Mass Accuracy Score	Isotopic Distribution Score	MS2 Score	XIC Score	Metabolite Name	Accession Number	Molecular Formula	Molecular Weight	Retention Time (min)	M. Gland: 090115P5BmouseMgland1_150904203809.raw	M. Gland: 090115P5BmouseMgland2_150904215317.raw	M. Gland: 090115P5BmouseMgland3_150904230800.raw	Breast Tumor: 090115P5BmousebreasttumorI_150904161622.raw	Breast Tumor: 090115P5BmousebreasttumorII_150904173129.raw	Breast Tumor: 090115P5BmousebreasttumorIII_150904184607.raw
0.786	0.29	4.73	0.89	0.96	Threonic acid_RT1 (+1)	HMDB:HMDB00943	$C_4H_8O_5$	136.0	3.85	9.34	9.42	9.44	8.92	9.62	8.83
0.981	0.96	5.00	1.00	0.97	Niacinamide_RT2 (+2)	HMDB:HMDB01406	$C_6H_6N_2O$	122.0	9.60	10.1	10.1	10.1	10.7	10.7	10.7
					Cluster of DL-Phenylalanine_RT2	CASNO:150-30-1									
0.979	0.98	5.00	1.00	0.95	DL-Phenylalanine_RT2 (+11)	CASNO:150-30-1	$C_9H_{11}NO_2$	165.1	9.66	9.06	9.06	Missing ...	10.2	10.1	10.1
0.957	0.98	5.00	0.81	0.95	3,4-Dihydro-2H-1-benzopyran-2-one_RT2 (+7)	HMDB:HMDB36626	$C_9H_8O_2$	148.1	9.68	9.01	9.06	Missing ...	10.2	10.1	10.1
0.969	0.98	3.59		0.97	(-)-1-Methylpropyl 1-propenyl disulfide (+17)	HMDB:HMDB34426	$C_7H_{14}S_2$	162.1	17.08	6.13	6.20	Missing ...	8.47	8.49	8.46
0.968	0.93	5.00	1.00	0.94	5'-Methylthioadenosine (+4)	HMDB:HMDB01173	$C_{11}H_{15}N_5O_3S$	297.1	22.77	8.11	8.11	8.18	7.90	7.94	7.81
0.968	0.95	3.37	1.00	0.95	1-Methyladenosine_RT4 (+5)	HMDB:HMDB03331	$C_{11}H_{15}N_5O_4$	281.1	19.59	7.02	7.05	7.10	7.61	7.86	7.88
					Cluster of Uridine_RT2	HMDB:HMDB00296									
0.966	0.98	4.63	1.00	0.97	Uridine_R								8.73	10.3	10.2	10.1
0.933	0.98	4.63		0.97	5-(2'-Carb								8.04	9.70	9.62	9.57
					Cluster of											
0.964	0.94	5.00	1.00	0.97	Pantother								9.39	9.98	9.90	9.87
0.933	0.94	5.00		0.96	4-Hepten								9.30	9.84	9.75	9.72
					Cluster of											
0.964	0.96	4.49	1.00	0.94	5-Methylc								Missing ...	9.24	9.10	9.11
0.927	0.96	4.49		0.91	(±)-2-(1-M								Missing ...	9.17	9.10	9.11
0.963	0.94	3.18	0.99	0.93	Cytosine_								8.88	10.2	10.1	10.1
0.963	1.00	2.54		0.95	Ethyl 2,4-								8.29	7.42	7.51	7.55
0.962	0.94	4.27	1.00	0.94	Cytidine_								8.76	10.4	10.4	10.3

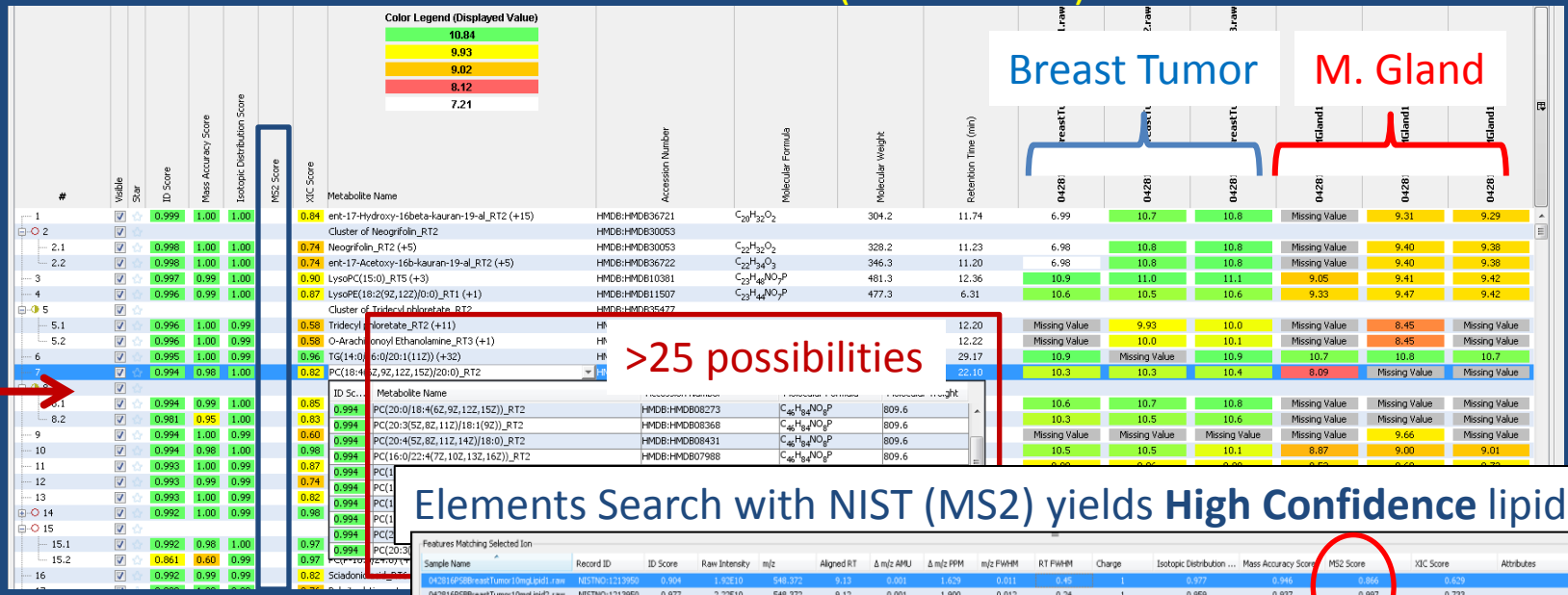


The mammary gland and the breast tumor have significantly different metabolite profiles

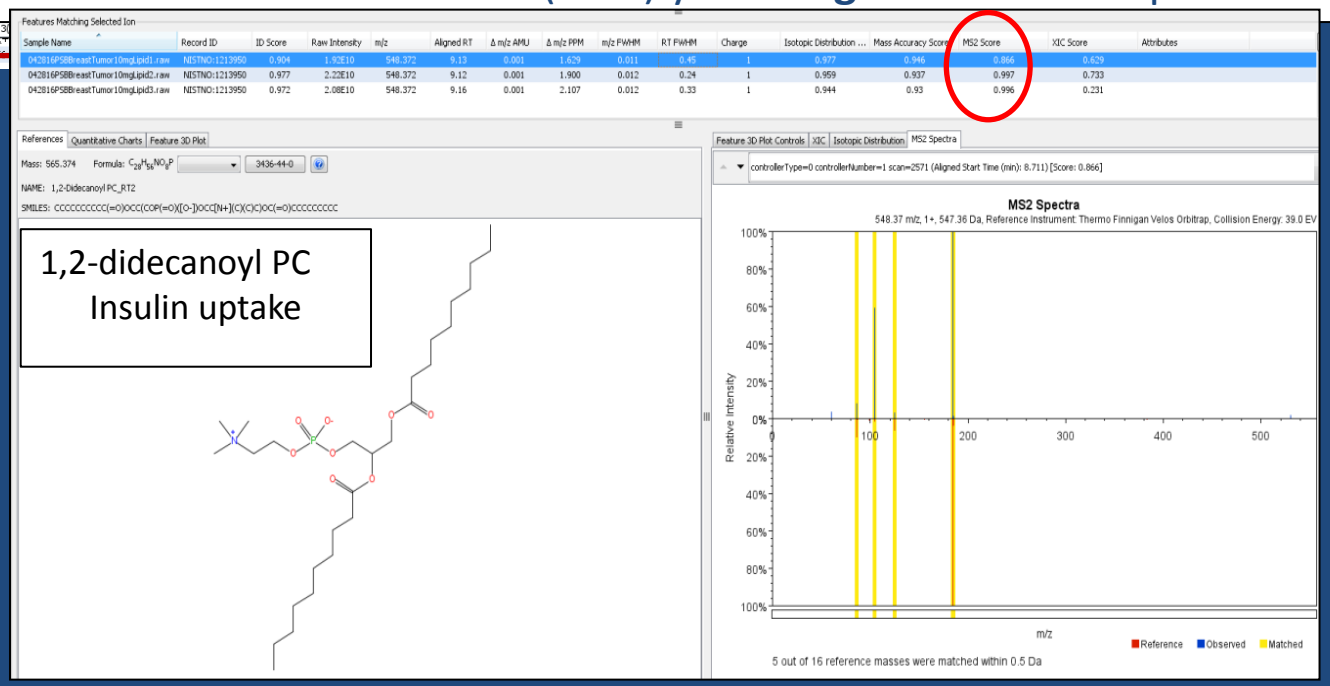
I prefer MS2 fragmentation data in order to trust an identification

- Even sub 1-2 ppm DB searches can be ambiguous in structure without MS2

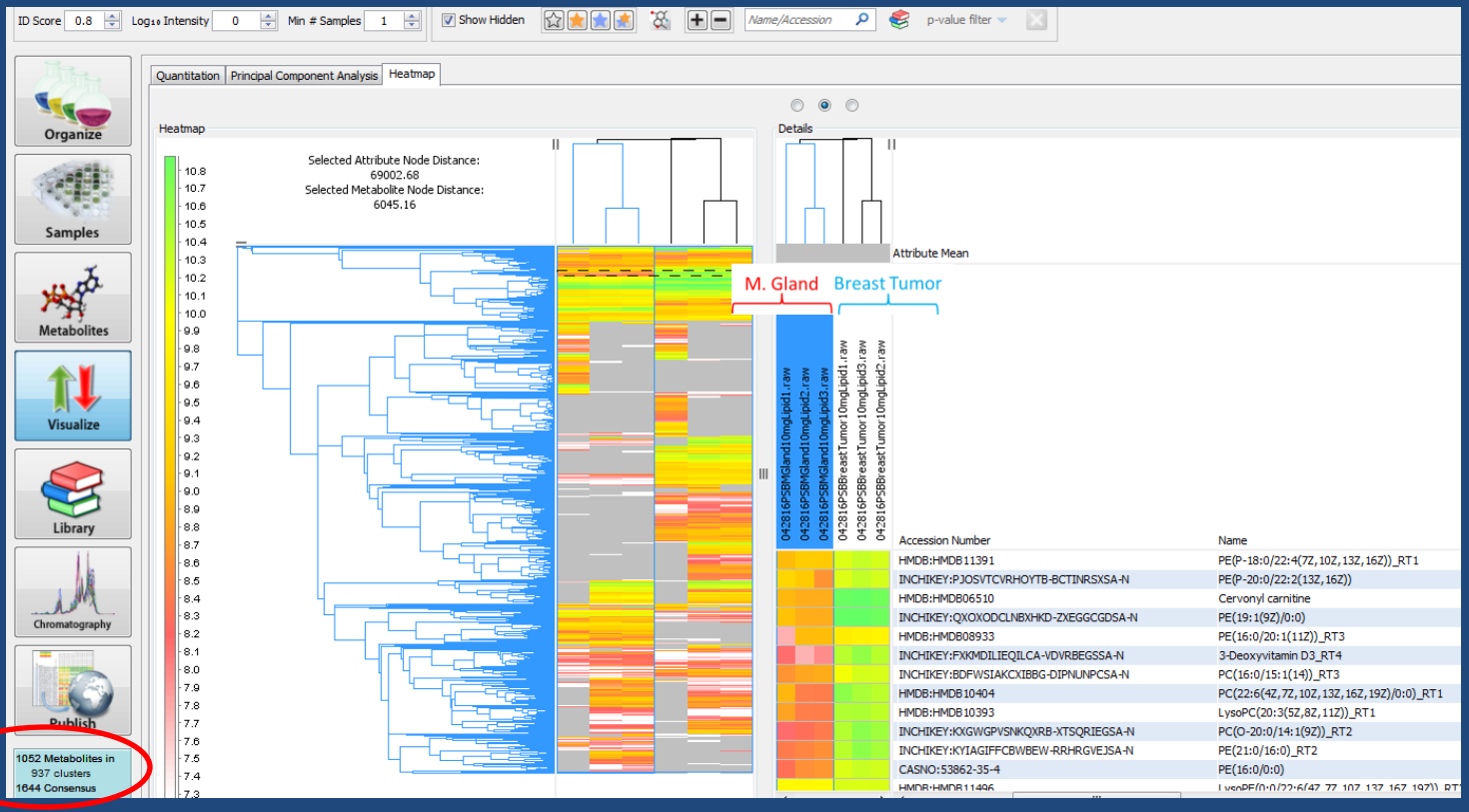
Elements Search with HMDB (no MS2 hits)



Elements Search with NIST (MS2) yields **High Confidence** lipid IDs



Elements MS and MS/MS mixed search



Library Statistics

NIST: 234,284 MS/MS spectra (open access)-MS Search, Elements

HMDB: 88,045 MS/MS (predicted and experimental), open access

mzCloud: ~200,000 filtered MS/MS spectra (Thermo)

Metlin: ~200,000 MS/MS spectra (XCMS online/Agilent)

How Do You Perform Informatics of Lipid Data?

Elements **NIST/HMDB IDs**

INCHIKEY:CORCYSWIDKFRAW-HIVNOOBXSA-N

↓

Convert to KEGG/HMDB using online tools (Fiehn)

↓

MetaboAnalyst Pathway Enrichment/Mapping

LipidSearch **lipid composition**

PC(16:0/18:1)

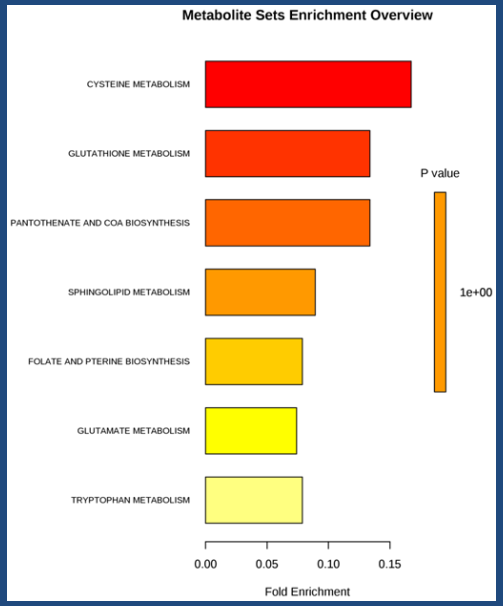
↓

Search LipidMaps for LipidMaps ID

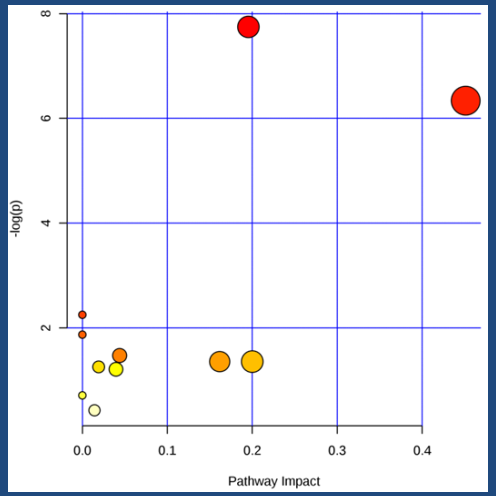
↓

LipidMaps ID links to HMDB/KEGG

Pathway Enrichment



Pathway Mapping

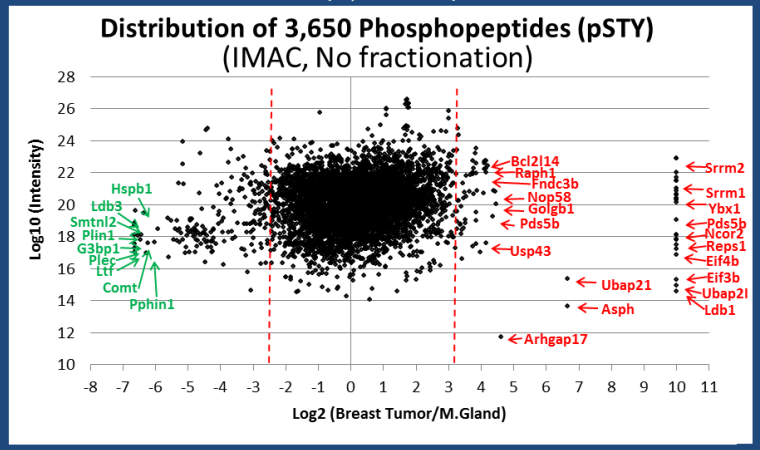


Identified Pathways

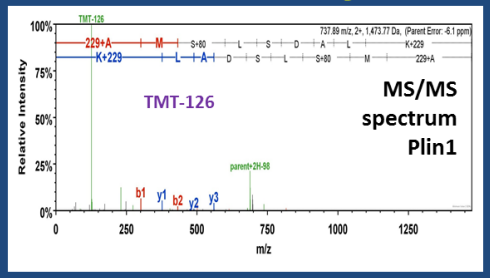
Pathway Name	Total	Hit	p	-log(p)	Holm p	FDR	Impact
Sphingolipid metabolism	21	4	0.0069634	4.9671	0.571	0.571	0.04261
Glycerophospholipid metabolism	30	3	0.0292	2.2738	1.0	1.0	0.25525
Linoleic acid metabolism	6	1	0.20828	1.5689	1.0	1.0	0.0
alpha-Linolenic acid metabolism	9	1	0.2958	1.2181	1.0	1.0	0.0
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	14	1	0.42104	0.86504	1.0	1.0	0.0439
Pantothenate and CoA biosynthesis	15	1	0.44332	0.81347	1.0	1.0	0.32653
Tryptophan metabolism	40	2	0.45603	0.7852	1.0	1.0	0.11562
Folate biosynthesis	16	1	0.46476	0.76623	1.0	1.0	0.0
Pentose and glucuronate interconversions	16	1	0.46476	0.76623	1.0	1.0	0.2
Starch and sucrose metabolism	19	1	0.52434	0.64562	1.0	1.0	0.03958
Glutathione metabolism	26	1	0.63919	0.44756	1.0	1.0	0.07824
Arachidonic acid metabolism	36	1	0.75748	0.27776	1.0	1.0	0.0
Drug metabolism - cytochrome P450	56	1	0.8914	0.11497	1.0	1.0	0.01429

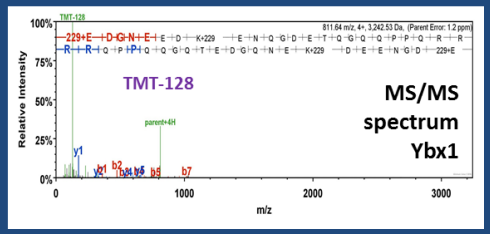
Serial-Omics on Mouse Breast Tumor vs Mammary Gland Tissue

P53-/-, Brca1 -/-

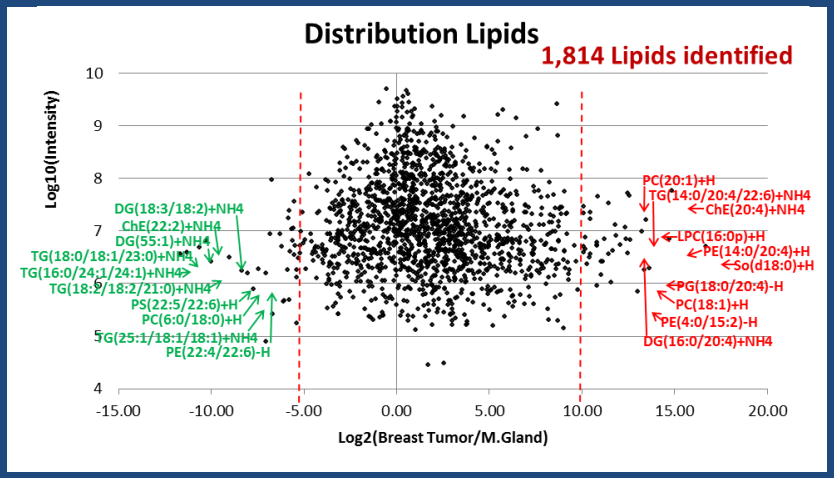


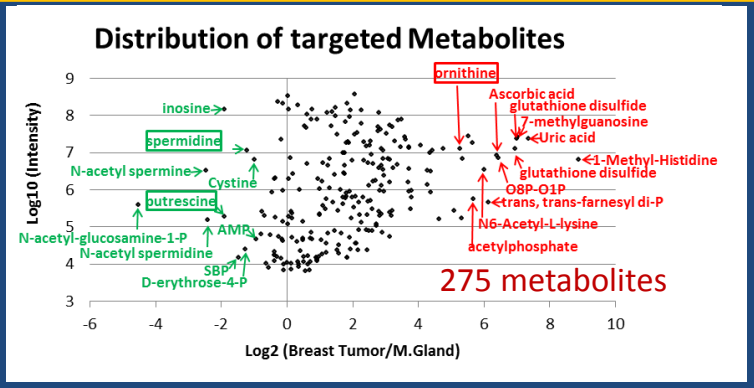
TMT Labeling

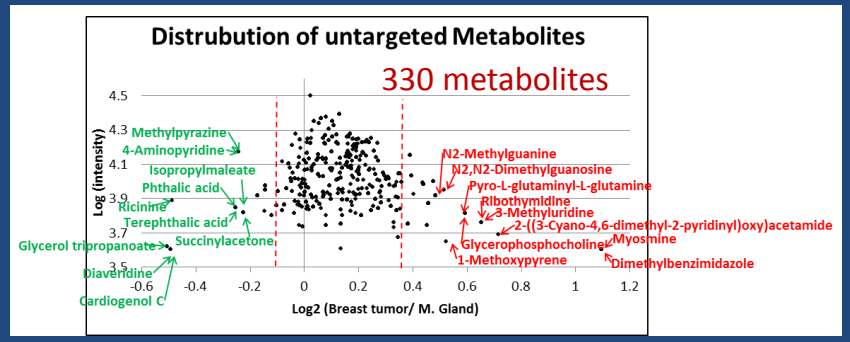




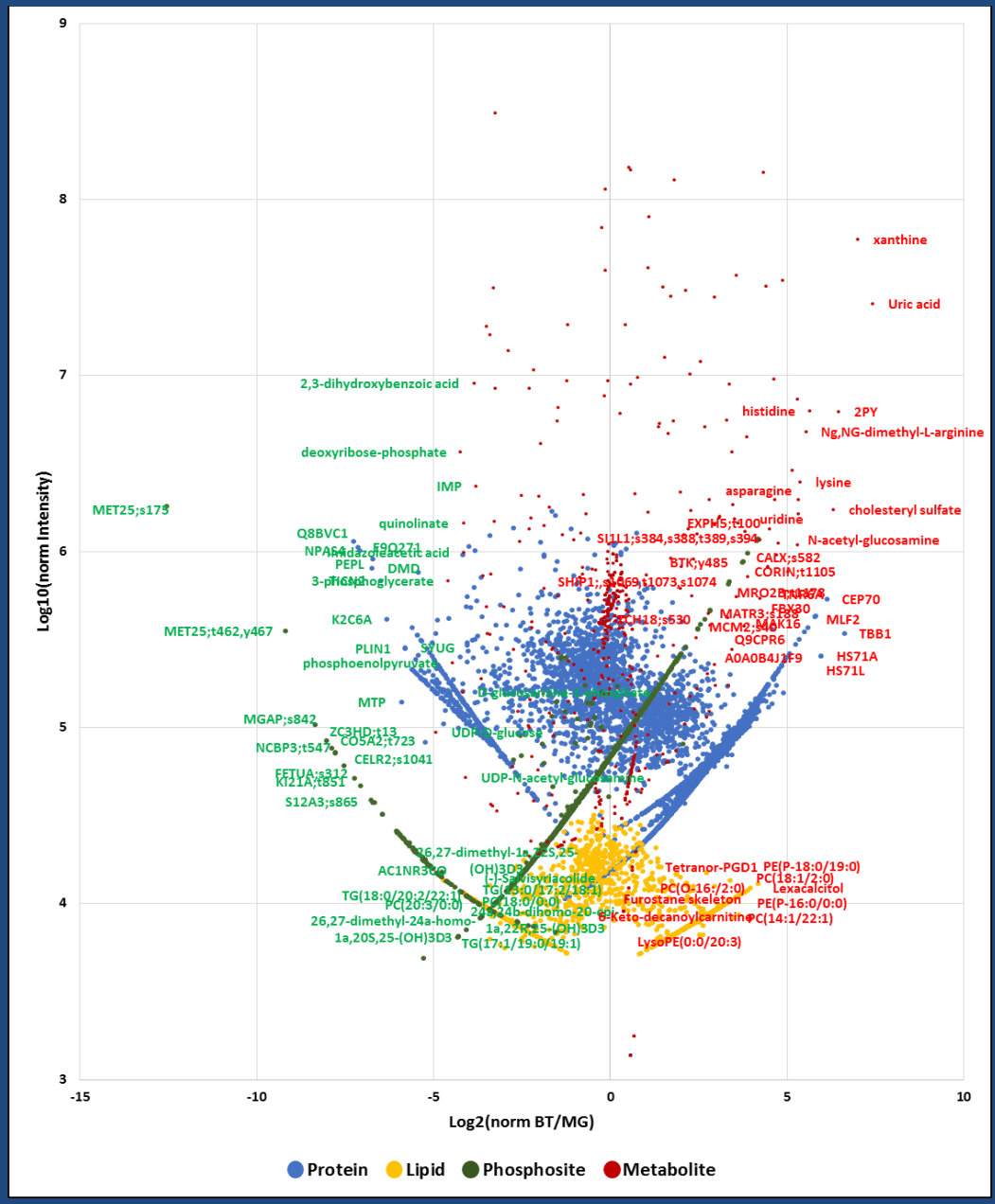
30 mg tissue







Integration of *Serial-Omics* Data

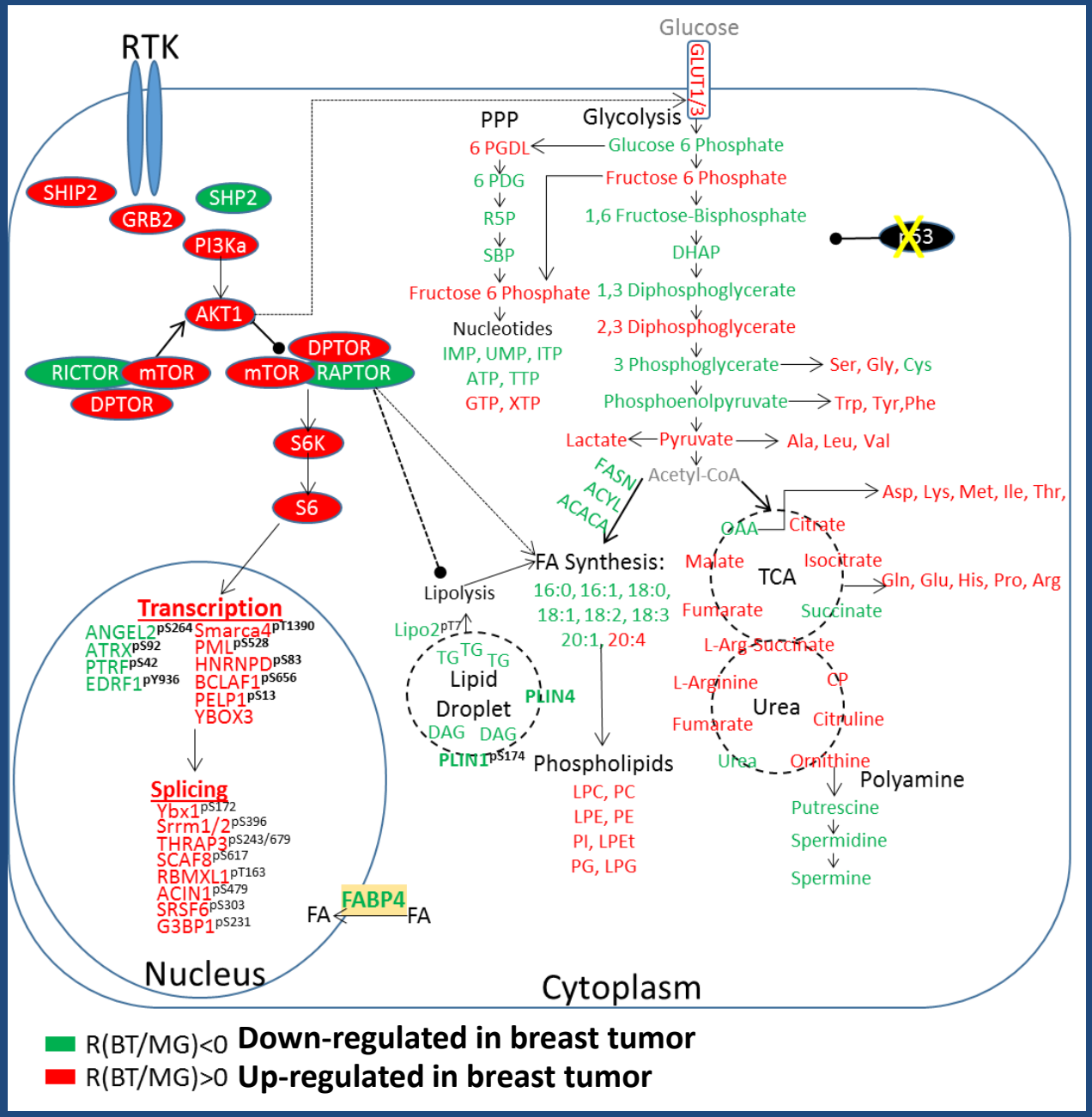


10 mg tissue

P53-/-, Brca1-/-

1,550 lipids
750 metabolites
7,500 proteins
1,071 unique phosphosites

Serial-Omics Integrated Pathway Map (Lipidomics, Metabolomics and Proteomics)



R(BT/MG)<0 **Down-regulated in breast tumor**

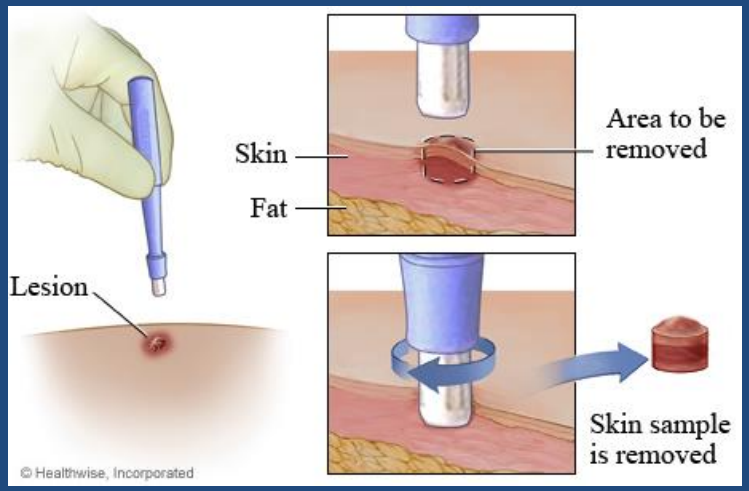
R(BT/MG)>0 **Up-regulated in breast tumor**

Mouse breast tumor

P53-/-, Brca1-/-

Our Goal is To Perform *Tri-Omics* from Single Tumor Biopsies

Typical Needle Biopsy



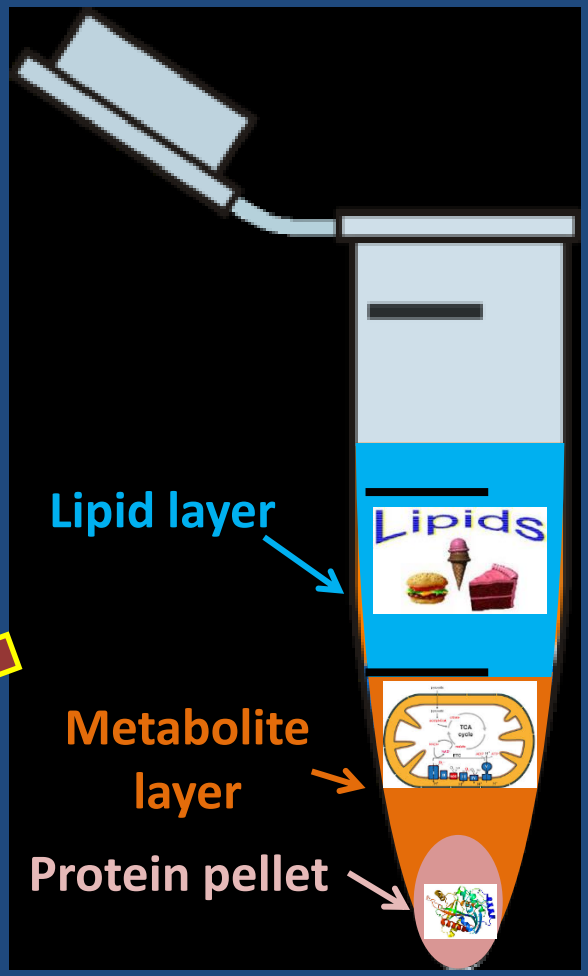
As little as 5-10 mg starting material

Labeling ($^{13}C/^{15}N$)

TMT

Methyl tert-butyl ether (MTBE)

Mainly used for lipid extractions



Serial-Omics

LC-MS/MS

Metabolomics, Lipidomics and Phosphoproteomics)

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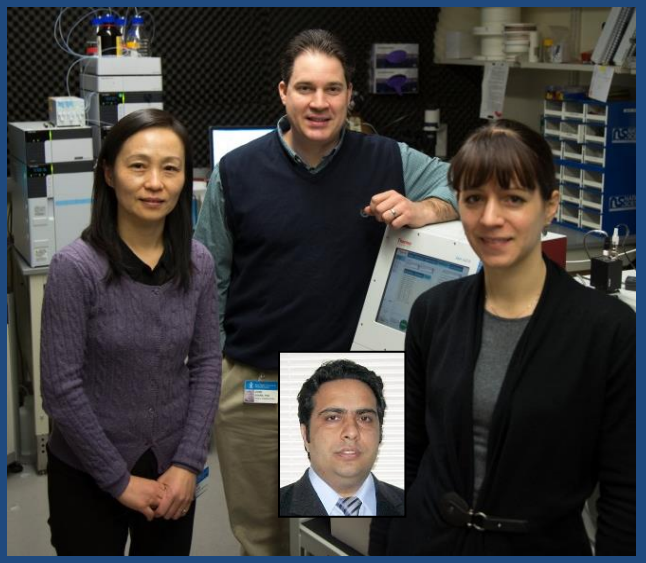

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